

# Inclusion of *Opuntia stricta* (Haw.) in sheep diets affects nutrition and the physicochemical characteristics of the rumen content

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**ABSTRACT** - The aim of this study was to evaluate the effects of the inclusion of the cactus *Opuntia stricta* (Haw.) in sheep diets on the feed intake, digestibility, fermentation, and physicochemical characteristics of the ruminal digesta. Five sheep cannulated in the rumen (61.5±9.5 kg body weight) were assigned in a Latin square design (5×5), with five diets and five experimental periods of 21 days each. The first 14 days were the adaptation period, and data were collected over the following seven days, making the total duration of the experiment 105 days. The diets included a control diet and four diets containing cactus at 121, 245, 371, and 500 g/kg of dry matter (DM). The diets had a forage:concentrate ratio of 65:35. The inclusion of cactus increased the DM intake and non-fiber carbohydrates, but reduced the neutral detergent fiber intake. It also increased the apparent digestibility of the DM, reduced the digesta density 4 h after feeding, and increased the production of ruminal fluid foam. The inclusion of cactus quadratically affected the DM rumen turnover, with the lowest value observed in the 336.5 g/kg cactus diet. The DM ruminal disappearance rate increased with the inclusion of cactus to the diets and quadratically affected the ruminal pH, with the highest value found in the 150 g/kg cactus diet. The concentrations of short-chain fatty acids (SCFA) increased, but the acetate:propionate ratio decreased with the inclusion of cactus at 500 g/kg DM. Taken together, our findings indicate that the evaluated spineless cactus can be added to sheep diets up to the level of 500 g/kg DM. The inclusion of *O. stricta* (Haw.) improves feed intake, DM digestibility, and SCFA and modifies the physicochemical characteristics of the ruminal digesta.

**Keywords:** degradation rate, feed evaluation, ruminal ammonia, rumen metabolism

## 1. Introduction

Arid and semi-arid regions present long periods of drought, which have become increasingly extended due to climate change (Ben Salem et al., 1996; Siqueira et al., 2017; Pinho et al., 2018). Drought conditions result in producers using alternative feed for livestock production. One feed alternative is the spineless cactus, which is rich in non-fiber carbohydrates (NFC, 640-710 g/kg dry matter (DM)) and water, making it an excellent alternative source of water and energy and enabling a reduction in the free water intake by animals (Stintzing and Carle, 2005; Abidi et al., 2009). Spineless cactus has a low content of neutral detergent fiber (NDF; 200-280 g/kg DM), which are usually related to

the laxative effect observed in ruminants fed exclusively or a large amount of this food. In this sense, cactus must be mixed with sources of effective fiber in ruminant diets, such as hay and straw (Batista et al., 2009; Cordova-Torres et al., 2015).

The carmine cochineal insect (*Dactylopius* sp.) is considered the main pest that infests cactus (Oliveira et al., 2010), leading to the search for more resistant cactus varieties that can be used as feed, such as *Opuntia stricta* (Haw.). This variety requires low soil fertility and, thus, has great cultivating potential in different environments (Cavalcanti et al., 2008). However, studies evaluating the use of *O. stricta* (Haw.) and its effects on the characteristics of the rumen content when added to ruminant diets are not conclusive. In a recent study, the use of cactus presented challenges over the histomorphometry of the ruminal epithelium in lambs and was possibly associated with changes to the rumen fermentation profile (Silva et al., 2020).

The physicochemical characteristics of the ruminal content (i.e., density, production of ruminal fluid foam) may be used as indicators of the alteration of the rumen fermentative profile and possibly metabolic disorders. However, few studies have reported physicochemical characteristics as indicators of metabolic disorders (Cannas et al., 2003; Clauss et al., 2016). Thus, the present study aimed to evaluate the effects of the inclusion of *O. stricta* (Haw.) in sheep diets on the feed intake, digestibility, fermentation, and physicochemical characteristics of the ruminal digesta.

## 2. Material and Methods

The experiment was carried out in accordance with the principles established by the institutional animal ethics committee (case number 065/2016).

Five adult sheep that were castrated and cannulated in the rumen (61.5±9.5 kg body weight (BW)) were distributed in a Latin square design (5×5), with five treatments and five experimental periods of 21 days each. The first 14 days were the adaptation period and the following seven days were for data collection, making the total duration of the experiment 105 days. Each animal was treated against endoparasites and ectoparasites and then housed in individual stalls equipped with individual drinking and feeding troughs.

Five experimental diets were used: a control diet composed of only Buffel hay (*Cenchrus ciliaris* L.) as forage (ground to pass through a 4-mm screen) and four other diets that included *O. stricta* (Haw.) at different concentrations (121, 245, 371, and 500 g/kg DM). The cactus was two years old at the time of cutting and came from an irrigated and fertilized area. Before feeding, the cactus was chopped to reach a particle size of approximately 1 cm.

The diets (Table 1) had a forage:concentrate ratio of 65:35 and were formulated to be isoproteic and to meet the requirements of the castrated male sheep for a daily gain of 100 g (NRC, 2007).

Feed was supplied twice a day, at 8:00 (60%) and at 4:00 h (40%), and the diet was offered as a complete mixed ration. The orts were weighed daily, and the offer was adjusted based on measurements from the previous day, allowing for orts of approximately 20%.

From days 8 to 10 during each experimental period, 20% feces, orts, and the ingredients composing the diets were sampled. These samples were homogenized to obtain a composite sample for each animal per period. The material was pre-dried in a forced-ventilation oven at 55 °C for 72 h and milled with Willey-type knives using a sieve screen with 1-mm mesh for orts and ingredients and 2-mm mesh for feces.

The samples were analyzed in accordance with the protocols described by the Association of Official Analytical Chemists (AOAC, 1997) for DM (method 930.15), crude protein (CP, method 954.01), ether extract (EE, method 920.39), and ash (method 942.05). The measurement of NDF was performed with an ANKOM fiber analyzer (ANKOM200 Fiber Analyzer; ANKOM Technology Corporation, Fairport, NY, USA). The NDF was corrected for ash and nitrogen by incinerating the neutral detergent digestion residues in a muffle oven at 600 °C for 4 h, and the correction for protein was performed by

the neutral detergent insoluble protein method (INCT-CA N method -004/1) based on the protocol described by Detmann (2012).

Ruminal fluid samples were collected during the first two days of the sampling period and were performed at 4-h intervals. On the first day, the ruminal fluid was collected before feeding (0 h) and 4 and 8 h after the morning feeding. On the second day, the ruminal fluid was collected 2 and 6 h after the morning feeding. The ruminal fluid samples were collected from four different points in the ventral region of the rumen and were subsequently homogenized. The digesta was filtered through four layers of cheesecloth, the liquid was homogenized immediately, and the pH was measured by direct reading with a digital potentiometer (Handy lab 12; Xylem Analytics Germany Sales GmbH & Corporation, Weilheim, KG, Germany).

After the pH measurement, a 20-mL aliquot was conditioned in a flask containing 2 mL of metaphosphoric acid (20%) and stored at  $-20\text{ }^{\circ}\text{C}$  for the subsequent determination of short-chain fatty acids (SCFA). An additional 20 mL of acid-free liquid was stored in another flask to measure the ruminal ammoniacal nitrogen (RAN). For the RAN determination, the samples were thawed, and 1 mL of trichloroacetic acid was added for each 6 mL of liquid. The samples were centrifuged at  $1000 \times g$  for 10 min and subjected to distillation, as described by Detmann (2012) (INCT-CA N-007/1).

The ruminal liquid samples were thawed and centrifuged at  $13,000 \times g$  for 13 min to quantify the SCFA concentration. Sample readings were performed in a high-performance liquid chromatographer (SHIMADZU, SPD-10A VP, Japan) coupled to a UV detector, using a wavelength of 210 nm and column HPX-87H, with dimensions of 30 cm  $\times$  4.5 mm diameter, a flow rate of 0.8 mL/min, pressure of 70 kgf, with the mobile phase of water in 0.05 MM of sulfuric acid, and the injected volume of 20  $\mu\text{L}$ .

For the analysis of the physicochemical characteristics of the ruminal digesta, rumen emptying was performed on the last two days of the collection period. The rumen was emptied before the morning feeding (0 h) on the first day and 4 h after the morning feeding on the second day.

**Table 1 - Proportion of ingredients and chemical composition of experimental diets**

Item	Inclusion of <i>Opuntia stricta</i> (Haw.) (g/kg of DM)				
	Control	121	245	371	500
Ingredient (g/kg DM)					
Ground corn	199.9	172.7	173.4	175.2	177.0
Soybean meal	132.0	166.6	168.3	170.0	170.7
Buffel hay	649.8	525.1	397.8	267.9	135.3
<i>O. stricta</i>	0.0	121.2	244.9	371.2	500.0
Urea	7.4	3.7	4.7	4.8	5.8
Ammonium sulfate	0.8	0.4	0.5	0.5	0.6
Mineral mix	10.1	10.2	10.3	10.4	10.5
Chemical composition (g/kg)					
Dry matter (DM)	918.4	529.4	369.7	282.7	227.9
Organic matter	937.4	924.9	913.4	901.7	889.7
Ash	61.8	74.6	86.1	97.7	109.6
Crude protein	147.2	149.0	150.6	149.5	150.8
Ether extract	21.9	21.3	21.5	21.7	22.0
aNDFn	483.3	429.8	375.8	320.9	264.7
NFC	285.9	325.2	366.0	410.1	453.0
Total carbohydrates	769.2	755.1	741.8	731.0	717.6

aNDFn - neutral detergent fiber with thermolabile amylase corrected for ash and nitrogen; NFC - non-fiber carbohydrates.

The total ruminal content was obtained by weighing the total digesta, after which this material was filtered through four layers of cheesecloth, separating the fluid fraction from the solid fraction. To measure the ruminal fluid foam production, 80 mL of the fluid fraction was placed in a 100 mL beaker, and after 5 min, the amount of ruminal fluid foam produced was measured in mL and converted to cm<sup>3</sup> (modified from Pressey et al., 1963).

For the estimation of total carbohydrates (TC, equation 1) and NFC (equation 2), the equations recommended by Sniffen et al. (1992) were applied along with the NDF with thermolabile amylase corrected for ash and nitrogen (aNDFn):

$$TC = 1000 - (CP, g + EE, g + Ash, g) \quad (1)$$

$$NFC = 1000 - (CP, g + aNDFn, g + EE, g + Ash, g) \quad (2)$$

The data for the calculation of the nutrient intake were determined from the measurements of the feed offered and theorts taken during the first three days of the collection period, as well as the data on feed composition.

For the apparent nutrient digestibility assay, the total fecal collection was performed for a period of 72 h during the first three days of the collection period, with the aid of collection bags. Every 12 h, the pouches were emptied and the feces were pre-dried, ground, and analyzed. The calculation of the apparent digestibility coefficient (DC) was performed based on equation 3.

$$DC \text{ nutrient (g/kg)} = \frac{\text{nutrient intake (g)} - \text{feces of nutrient (g)}}{\text{nutrient intake (g)}} \times 1000 \quad (3)$$

To obtain the density of the digesta, 1 L of the total rumen content (composed of the mixture of the fluid and solid fraction of the ruminal content) was weighed in a test tube, and the following equation (4) was applied:

$$D = \frac{M}{V} \quad (4)$$

in which D is the density of the digesta, M is the mass in kg, and V is the volume in L.

The DM rumen turnover (DMRT; h) and the DM ruminal disappearance rate (DMDR; %/h) were calculated from the relationship between the rumen content (kg DM) and feed intake (kg DM/h) based on the equations proposed by Cannas et al. (2003).

The variables were analyzed using the PROC REG procedure in the SAS software program (Statistical Analysis System, version 9.2), adopting 0.05 as the critical level of probability or tendency ( $P < 0.10$ ) for a type I error and using the mathematical model described below (equation 5):

$$Y_{ijk} = \mu + \tau_i + \alpha_j + \beta_k + \varepsilon_{ijk} \quad (5)$$

in which  $\gamma_{ijk}$  is the dependent variable measured in animal  $j$  that was subjected to treatment  $i$  in period  $k$ ,  $\mu$  is the overall mean,  $\tau_i$  is the fixed effect of treatment  $i$ ,  $\alpha_j$  is the random effect of animal  $j$ ,  $\beta_k$  is the random effect of period  $k$ , and  $\varepsilon_{ijk}$  is the random error.

After analyzing the variance effects, the significance of the linear and quadratic effects of the inclusion of cactus in Buffel hay was evaluated. The ruminal fermentation variables (pH, RAN, and SCFA) were analyzed considering the effects of measurements over time.

### 3. Results

When we included cactus in the sheep diet up to 500 g/kg DM (Table 2), the DM intake increased from 1,250.7 to 1,748.2 g/d and from 55.5 to 81.4 g/kg of metabolic weight ( $P < 0.05$ ). The inclusion of cactus also increased the ash intake (from 76.4 to 198.5 g/d;  $P < 0.05$ ), NFC intake (from 363.5 to

823.7 g/d;  $P < 0.05$ ), and TC intake (from 952.5 to 1,272.3;  $P < 0.05$ ), as well as the digestible organic matter (DOM) intake (from 818.6 to 1,316.8 g/d;  $P < 0.05$ ). However, the aNDFn intake decreased from 589.0 to 448.6 g/d ( $P < 0.05$ ) with the inclusion of cactus in the diets up to 500 g/kg DM.

The apparent digestibility of DM, OM, NFC, and TC linearly increased with the inclusion of cactus in the diets up to 500 g/kg DM ( $P < 0.05$ ). The digestibility of CP and aNDFn was not influenced by the inclusion of cactus in the diets ( $P > 0.05$ ; Table 2).

The inclusion of cactus up to 500 g/kg DM decreased the rumen content before feeding (from 8.6 to 7.1% BW) and 4 h after feeding (from 10.7 to 8.5% BW;  $P < 0.05$ ). The density of the digesta decreased with the inclusion of cactus in the diets 4 h after feeding ( $P < 0.05$ ). Before feeding (0 h), we verified a tendency of reduction in the density of the digesta (from 0.964 to 0.901 g/cm<sup>3</sup>;  $P < 0.10$ ). The ruminal fluid foam production increased before feeding (from 0.280 to 0.860 mm) and 4 h after feeding (from 0.300 to 0.680 mm) with the inclusion of cactus in the diets up to 500 g/kg DM ( $P < 0.05$ ; Table 3).

The inclusion of cactus in the diets quadratically affected the DMRT, with a minimum point of 7.15 h at 336.5 g/kg ( $P < 0.05$ ; Table 3). The DMDR linearly increased as more cactus was added to the diets, ranging from 8.13%/h in the control diet to 15.67%/h in the 500 g/kg DM diet ( $P < 0.05$ ).

The pH was quadratically affected by the inclusion of cactus, presenting a maximum value of 6.39 when the animals were fed 245 g/kg DM of cactus in the diet ( $P < 0.05$ ; Table 4). The RAN was also quadratically affected, with a minimum value of 10.47 mg/dL, when the animals were fed 245 g/kg DM of cactus in the diet ( $P < 0.05$ ). The inclusion of cactus increased the concentrations of acetate (from 38.21 to 49.27 mMol/L), propionate (from 10.66 to 19.63 mMol/L), butyrate (from 0.62 to 1.07 mMol/L), and SCFA (from 49.49 to 69.89 mMol/L;  $P < 0.05$ ). The acetate:propionate ratio (A:P) decreased (from 3.65 to 2.67) with the inclusion of cactus up to 500 g/kg DM ( $P < 0.05$ ).

**Table 2** - Intake and apparent digestibility of nutrients by sheep fed *Opuntia stricta* (Haw.)

Variable	Inclusion of cactus (g/kg of DM)					SEM	P-value	
	Control	121	245	371	500		L	Q
Intake (g/kg of LW <sup>0.75</sup> )								
Dry matter	55.5	68.3	78.0	76.9	81.4	2.79	0.0065	0.219
Intake (g/d)								
Dry matter	1250.7	1487.3	1759.8	1671.8	1748.2	0.067	0.0251	0.225
Organic matter	1174.3	1370.9	1603.9	1503.4	1549.7	0.058	0.0555	0.213
Ash	76.4	116.5	155.9	168.4	198.5	0.010	<0.0001	0.368
Crude protein	193.4	236.4	262.0	239.5	237.3	0.009	0.1892	0.092
aNDFn	589.0	582.5	624.8	481.0	448.6	0.024	0.0395	0.265
NFC	363.5	518.3	675.8	742.9	823.7	0.040	<0.0001	0.271
TC	952.5	1100.8	1300.6	1223.8	1272.3	0.048	0.0463	0.253
DOM	818.6	1061.1	1304.7	1247.1	1316.8	0.058	0.0086	0.162
Digestibility (g/kg)								
Dry matter	672.3	730.9	779.8	808.2	800.6	15.17	0.0044	0.184
Organic matter	694.7	749.0	796.0	827.3	820.9	14.58	0.0029	0.198
Crude protein	786.5	837.6	845.7	846.2	837.8	9.82	0.1518	0.160
aNDFn	585.3	619.0	677.9	707.9	690.4	18.68	0.0581	0.430
NFC	815.6	846.1	881.7	895.9	893.6	9.99	0.0059	0.237
TC	673.7	728.1	784.2	823.0	817.4	16.0	0.0015	0.216

LW<sup>0.75</sup> - metabolic weight; aNDFn - neutral detergent fiber with thermostable amylase corrected for ash and nitrogen; NFC - non-fiber carbohydrates; TC - total carbohydrates; DOM - digestible organic matter; SEM - standard error of the mean; L - linear effect; Q - quadratic effect.  $P < 0.05$ .

The pH as a function of the collection times (Figure 1) was quadratically affected by the inclusion of cactus ( $P < 0.05$ ), with a minimum point at 4 h after feeding for all diets evaluated. The RAN content as a function of the sampling time was also quadratically affected by the inclusion of cactus (Figure 2;  $P < 0.05$ ), with a maximum point at 4 h after feeding (33.05 mg/dL). Diets including cactus presented similar patterns and lower levels of RAN as a function of time (Figure 2).

#### 4. Discussion

The inclusion of *O. stricta* affected the nutrient intake, digestibility, production of SCFA, and the physicochemical characteristics of ruminal digesta in sheep.

Cactus, in general, presents high ruminal degradability and palatability, and thus its inclusion possibly affects digestibility and feed intake (Costa et al., 2016). The carbohydrate composition of *O. stricta* may

**Table 3 - Physicochemical characteristics of rumen of sheep fed *Opuntia stricta* (Haw.) before (0 h) and after feeding (4 h)**

Variable	Inclusion of cactus (g/kg of DM)					SEM	P-value	
	Control	121	245	371	500		L	Q
Ruminal content (% LW)								
0 h	8.57	7.89	7.45	6.76	7.13	0.298	0.0338	0.348
4 h	10.70	10.26	8.97	8.39	8.46	0.380	0.0027	0.367
Digesta density (g/cm <sup>3</sup> )								
0 h	0.978	0.941	0.924	0.940	0.910	0.011	0.0765	0.583
4 h	0.964	0.934	0.902	0.922	0.901	0.010	0.0097	0.203
Ruminal fluid foam (mm)								
0 h	0.280	0.600	0.440	0.800	0.860	0.065	0.0006	0.966
4 h	0.300	0.380	0.460	0.480	0.680	0.046	0.0075	0.597
DMRT (h)	12.91	9.23	7.34	6.95	7.54	0.626	0.0007	0.011*
DMDR (%/h)	8.13	11.54	14.21	15.10	15.67	0.008	0.0035	0.097

DM - dry matter; LW - live weight; SEM - standard error of the mean; L - linear effect; Q - quadratic effect; DMRT - DM rumen turnover; DMDR - dry matter ruminal disappearance rate.  $P < 0.05$ .

\*  $\gamma = 12.81 - 0.033x + 0.00005x^2$ ,  $R^2 = 0.99$ .

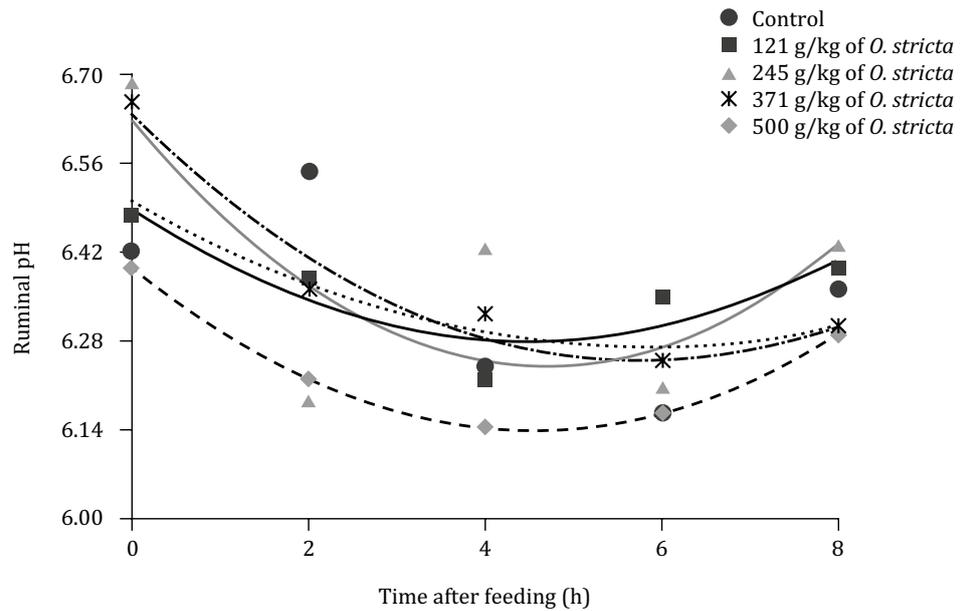
**Table 4 - Characterization of ruminal fermentation of sheep fed *Opuntia stricta* (Haw.)**

Variable	Inclusion of cactus (g/kg of DM)					SEM	P-value				
	Control	121	245	371	500		Treatment (Tr)		Time (T)		Tr × T
							L	Q	L	Q	
pH	6.35	6.37	6.39	6.38	6.24	0.057	0.1536	0.0427*	0.0021	0.0001	0.3690
RAN (mg/dL)	20.09	15.64	10.47	13.48	14.86	1.702	0.0005	<0.0001**	0.4624	0.0031	0.0007
Acetate (mMol/L)	38.21	45.15	43.77	45.11	49.27	2.324	0.0007	0.8012	0.0144	<0.0001	0.2134
Propionate (mMol/L)	10.66	14.23	14.45	16.35	19.63	1.211	<0.0001	0.6683	<0.0001	<0.0001	0.1886
Butyrate (mMol/L)	0.62	0.89	0.88	0.96	1.07	0.059	<0.0001	0.3050	0.1782	<0.0001	0.2984
SCFA (mMol/L)	49.49	60.23	59.10	62.41	69.89	3.428	<0.0001	0.9241	0.0008	<0.0001	0.2580
A:P	3.65	3.23	3.23	3.15	2.67	0.175	<0.0001	0.6719	<0.0001	<0.0001	0.0031

DM - dry matter; RAN - ruminal ammoniacal nitrogen; SCFA - short-chain fatty acids; A:P - acetate to propionate ratio; SEM - standard error of the mean; L - linear effect; Q - quadratic effect.

\*  $\gamma = 6.34 + 0.0006x - 0.000002x^2$ ,  $R^2 = 0.88$ .

\*\*  $\gamma = 20.22 - 0.055x + 0.00009x^2$ ,  $R^2 = 0.8$ .



SEM = 0.057; quadratic effect for all treatments as a function of time (i.e.,  $P < 0.05$ ).

Equations:

Control -  $\gamma = 5.50 + 0.078x - 0.006x^2$ ,  $R^2 = 0.38$ .

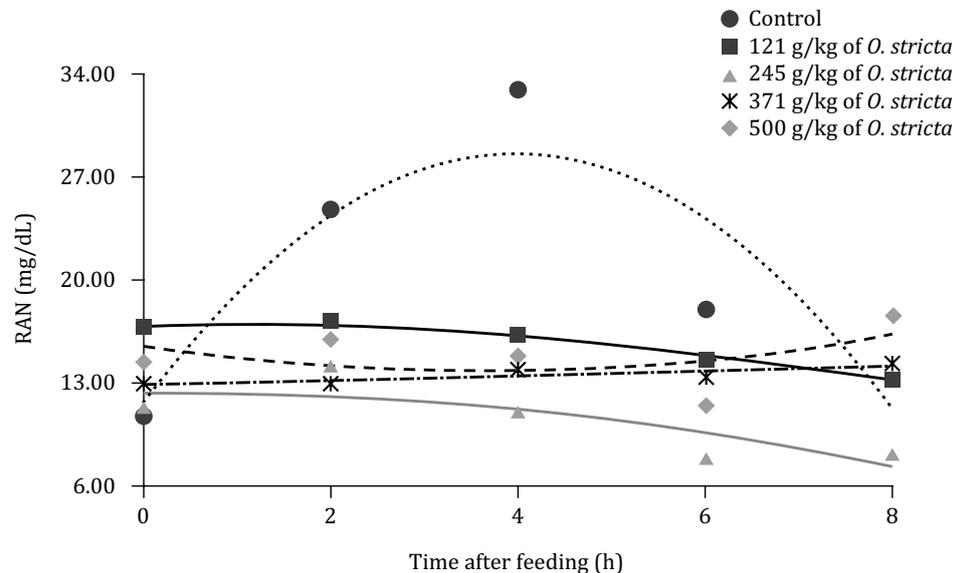
121 g/kg of *O. stricta* (Haw.) -  $\gamma = 6.49 - 0.093x + 0.01x^2$ ,  $R^2 = 0.78$ .

245 g/kg of *O. stricta* (Haw.) -  $\gamma = 6.63 - 0.166x + 0.017x^2$ ,  $R^2 = 0.56$ .

371 g/kg of *O. stricta* (Haw.) -  $\gamma = 6.63 - 0.135x + 0.018x^2$ ,  $R^2 = 0.95$ .

500 g/kg of *O. stricta* (Haw.) -  $\gamma = 6.39 - 0.113x + 0.012x^2$ ,  $R^2 = 0.99$ .

**Figure 1** - pH values of ruminal fluid of sheep subjected to diets containing *Opuntia stricta* (Haw.) as a function of collection times.



SEM = 1.702; quadratic effect for all diets as a function of time (i.e.,  $P < 0.05$ ).

Equations:

Control -  $\gamma = 11.72 + 8.58x - 1.081x^2$ ,  $R^2 = 0.80$ .

121 g/kg of *O. stricta* (Haw.) -  $\gamma = 16.97 + 0.089x - 0.070x^2$ ,  $R^2 = 0.96$ .

245 g/kg of *O. stricta* (Haw.) -  $\gamma = 12.27 + 0.078x - 0.088x^2$ ,  $R^2 = 0.66$ .

371 g/kg of *O. stricta* (Haw.) -  $\gamma = 12.93 + 0.102x - 0.006x^2$ ,  $R^2 = 0.64$ .

500 g/kg of *O. stricta* (Haw.) -  $\gamma = 15.48 - 0.913x + 0.126x^2$ ,  $R^2 = 0.18$ .

**Figure 2** - Ruminal ammoniacal nitrogen (RAN) content of sheep receiving diets containing *Opuntia stricta* (Haw.) as a function of time after feeding.

have influenced the ruminal fermentation parameters in the present study. The presence of pectin in the form of mucilage may modify the physicochemical characteristics of the ruminal digesta; according to Souza Filho et al. (2016), cactus presents from 5.4 to 5.6% of calcium pectate in DM. Although we did not evaluate the pectin content in the *O. stricta* used in the current study, this component is notable in the cactus composition and should be considered in future studies for a better understanding of the effects on ruminal digesta.

The variety of cactus evaluated herein presented higher NFC, ash, and DOM contents than the Buffel hay. Thus, the inclusion of cactus in diets may affect the quantity of these nutrients ingested, resulting in a greater intake of NFC, ashes, and DOM and a lower intake of aNDFn. Other researchers also found an increase in the DM intake in diets using cactus (Nefzaoui and Ben Salem, 2001). The increase in DM intake occurs mainly because of the palatability and high rate of passage of cactus, increasing the ruminal degradation rate that results in less filling of the rumen and thus increasing the intake rate (Agudelo, 2007; Costa et al., 2016).

Diets with high levels of cactus presented high ruminal degradability, which is directly related to low concentrations of acid detergent fiber and lignin (Andrade-Montemayor, 2005). The high ruminal degradability of diets with cactus possibly increased the digestibility of most nutrients evaluated in the present study. *Opuntia stricta* presents carbohydrates of high solubility in the rumen (Andrade-Montemayor et al., 2011). Additionally, the inclusion of this cactus in the diets reduces the content of aNDFn, which might have contributed to the increase in the apparent digestibility of DM, NFC, and TC in the diets.

The DM and NDF intake influences the ruminal content, since the higher the DM and NDF intake, the higher the ruminal content (Church, 1993). Diets that promote a lower NDF intake, such as those tested in the present study, have decreased ruminal contents owing to rapid ruminal emptying, either by substrate degradation or by passing to the next organs in the digestive tract (Silva et al., 2017). Additionally, the reduction in the ruminal content might have occurred because of the increase in NFC in the diets containing cactus (from 285 to 453 g/kg DM). The rapid degradation of NFC in the rumen may determine the increase in digestion and the rate of passage, promoting the lowest ruminal filling by the rapid emptying of the rumen, either by digestion or passage to other compartments in the gastrointestinal tract (Pinho et al., 2018).

The density of the digesta might be influenced by both the mass (g) and volume (cm<sup>3</sup>) of the rumen content. Considering that in all diets the volume was maintained at 1 L, the density reduced as a function of the reduction of the mass in the rumen content. The reduction in the mass in the rumen content with the inclusion of cactus may have occurred because of the increase in gas production and foaming, which would occupy spaces destined for digesta (Cavalcanti et al., 2008) and, thus, reduce the mass of the rumen content.

The increase in the ruminal fluid foam production probably occurred because of the presence of mucilage in the cactus, promoting an increase in the viscosity of the ruminal fluid and preventing the union of gas bubbles during fermentation (Sáenz et al., 2004). Another factor explaining this increase in foaming is the rapid degradation of protein and soluble carbohydrates in the rumen, which promotes the formation of a microbial digestive consortium (biofilm) that captures the gases produced by ruminal fermentation and, thus, generates ruminal fluid foam (Pinchak et al., 2005).

In the present study, the high DM intake and digestibility possibly influenced the high DMRT in diets including cactus. Turnover can be affected by factors such as feed intake, digestibility, rate of passage, density, and particle size (Van Soest, 1994; Allen, 1997; Dufreneix et al., 2019). Additionally, the increase in the turnover of diets with a high level of cactus may be related to the lower density of the digesta, which promotes the reduction of microbial activity and, consequently, affects the colonization of microorganisms in the substrate (Andrade et al., 2016).

The DMDR represents the disappearance of DM in the rumen, either by microbial degradation or by passage to the next digestive compartments (Church, 1993). As expected, the same factors that affect

DMRT also affect DMDR; therefore, the increase in DMDR is related to the increased degradability of most nutrients after the inclusion of cactus in the diets.

Ruminal microorganisms need an ideal pH range, which varies according to diet and time after feeding (Church, 1993; Oliveira et al., 2013). In the present study, we observed a quadratic pattern for pH values in the rumen, in which the highest value (6.39) occurred in the 150 g/kg cactus treatment, after which the pH decreased. Cactus has a high pectin content in relation to other forages that, when degraded, generates galacturonic and acetic acids, which are weak acids. Additionally, the mucilage and high ash content may promote increased saliva production by ruminants, which leads to ruminal tamponade (Andrade-Montemayor et al., 2011).

When assessing pH as a function of time, we verified a quadratic pattern. This was indicated by a decrease up to 4 h after feeding, followed by an increase, indicating the absence of ruminal acidosis. According to Van Soest (1994), a pH above 6.2 is interesting because it is associated with the time of colonization by microorganisms and, consequently, the degradation of the fiber. The pH values measured in the diets evaluated in the present study were below 6.2, indicating a satisfactory condition for fiber degradation with respect to this aspect.

The RAN concentration is dependent on the degradation rate of the protein source used and the balance between its production and use by ruminal microorganisms (Manella et al., 2002). At all levels of cactus inclusion evaluated, the RAN content met the minimum requirement for microbial growth, considering a minimum content of 5 mg/dL (Satter and Slyter, 1974).

When assessing RAN as a function of time, the peak (33.05 mg/dL) occurred in the control diet, which is a response to the differences between sources of protein present in the diets (soybean meal and urea). An increase in the colonization time of the microorganisms on the fibrous fraction of the substrate results in the accumulation of nitrogen in the rumen due to the imbalance between the degradation of the two nutrients (Wanapat and Kang, 2013). In the present study, a greater synchrony in the degradation of the protein and energy sources occurred in diets containing cactus. This balance may indicate differences in the efficiency of nitrogen use by ruminal microorganisms (Costa et al., 2016).

The increase in the production of acetate, propionate, and butyrate, as well as the total SCFA, is directly related to the high ruminal degradation rate of *O. stricta* (Lins et al., 2016). In addition, sheep fed cactus showed a higher DM intake, which provides a greater amount of substrate for the fermentation of ruminal microorganisms (Clauss et al., 2016). The acetate and butyrate contents are related to the high levels of pectin and soluble sugars, respectively, in cactus. However, the increase in propionate occurs via the fermentation of starch, which is also present in great quantities in cactus (Batista et al., 2009). The acetate:propionate ratio decreased, even with the increase in the concentration of these two acids, which is possibly caused by the higher formation of propionate to the detriment of acetate. Another component that possibly affects the SCFA content is the NDF composition in the diets evaluated (Van Soest, 1994). When we included cactus in the diet, the NDF level was reduced, and its fermentation possibly influenced the higher propionate levels and lower acetate levels in the animals fed more cactus.

Considering the nutritional characteristics of *O. stricta*, it is recommended for use in sheep feed because it promotes a better ruminal use of nutrients in the diet. However, further studies are required to evaluate the effects of *O. stricta* on milk and sheep meat quality, considering the importance of this feed as a nutritional strategy under arid and semiarid conditions.

## 5. Conclusions

*Opuntia stricta* can be included in the diets of sheep up to 500 g/kg DM. Its inclusion increases feed intake, dry matter digestibility, production of short-chain fatty acids, and modification of the physicochemical characteristics of the ruminal digesta.

## Conflict of Interest

The authors declare no conflict of interest.

## Author Contributions

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## References

- Abidi, S.; Ben Salem, H.; Vasta, V. and Priolo, A. 2009. Supplementation with barley or spineless cactus (*Opuntia ficus indica* f. *inermis*) cladodes on digestion, growth and intramuscular fatty acid composition in sheep and goats receiving oaten hay. *Small Ruminant Research* 87:9-16. <https://doi.org/10.1016/j.smallrumres.2009.09.004>
- Agudelo, J. C. C. 2007. Efecto de la utilización de arbóreas y arbustivas forrajeras sobre la dinámica digestiva en bovinos. *Revista Lasallista de Investigación* 4:40-50.
- Allen, M. S. 1997. Relationship between fermentation acid production in the rumen and the requirement for physically effective fiber. *Journal of Dairy Science* 80:1447-1462. [https://doi.org/10.3168/jds.S0022-0302\(97\)76074-0](https://doi.org/10.3168/jds.S0022-0302(97)76074-0)
- Andrade, S. F. J.; Batista, A. M. V.; Carvalho, F. F. R.; Lucena, R. B.; Andrade, R. P. X. and Lima Júnior, D. M. 2016. Fresh or dehydrated spineless cactus in diets for lambs. *Acta Scientiarum. Animal Sciences* 38:155-161. <https://doi.org/10.4025/actascianimsci.v38i2.29329>
- Andrade-Montemayor, H. 2005. Valoración nutritiva de los sistemas de alimentación y evaluación del uso de fuentes alternativas de proteína vegetal en la alimentación de caprinos. Thesis (D.Sc.). Universidad de Murcia, España.
- Andrade-Montemayor, H. M.; Cordova-Torres, A. V.; García-Gasca, T. and Kawas, J. R. 2011. Alternative foods for small ruminants in semiarid zones, the case of Mesquite (*Prosopis laevigata* spp.) and Nopal (*Opuntia* spp.). *Small Ruminant Research* 98:83-92. <https://doi.org/10.1016/j.smallrumres.2011.03.023>
- AOAC. Cuniff, P. 1997. Official methods of analysis of AOAC International. AOAC International.
- Batista, A. M. V.; Ribeiro Neto, A. C.; Lucena, R. B.; Santos, D. C.; Dubeux Júnior, J. B. and Mustafa, A. F. 2009. Chemical composition and ruminal degradability of spineless cactus grown in Northeastern Brazil. *Rangeland Ecology & Management* 62:297-301. <https://doi.org/10.2111/07-099R1.1>
- Ben Salem, H.; Nefzaouli, A.; Abdouli, H. and Orskov, E. R. 1996. Effect of increasing level of spineless cactus (*Opuntia ficus indica* var. *inermis*) on intake and digestion by sheep given straw-based diets. *Animal Science* 62:293-299. <https://doi.org/10.1017/S1357729800014600>

- Cannas, A.; Van Soest, P. J. and Pell, A. N. 2003. Use of animal and dietary information to predict rumen turnover. *Animal Feed Science and Technology* 106:95-117. [https://doi.org/10.1016/S0377-8401\(02\)00255-9](https://doi.org/10.1016/S0377-8401(02)00255-9)
- Cavalcanti, M. C. A.; Batista, A. M. V.; Guim, A.; Lira, M. A.; Ribeiro, V. L. and Ribeiro Neto, A. C. 2008. Consumo e comportamento ingestivo de caprinos e ovinos alimentados com palma gigante (*Opuntia ficus-indica* Mill) e palma orelha-de-elefante (*Opuntia* sp.). *Acta Scientiarum. Animal Sciences* 30:173-179. <https://doi.org/10.4025/actascianimsci.v30i2.4684>
- Church, D. C. 1993. *The ruminant animal: Digestive, physiology and nutrition*. Simon & Schuster, Englewood Cliffs.
- Clauss, M.; Stewart, M.; Price, E.; Pelson, A.; Savage, T.; Ekris, I. V. and Munn, A. 2016. The effect of feed intake on digesta passage, digestive organ fill and mass, and digesta dry matter content in sheep (*Ovis aries*): Flexibility in digestion but not in water reabsorption. *Small Ruminant Research* 138:12-19. <https://doi.org/10.1016/j.smallrumres.2016.03.029>
- Cordova-Torres, A. V.; Mendonza-Mendonza, J. C.; Bernal-Santos, G.; García-Gasca, T.; Kawas, J. R.; Costa, R. G.; Jacobo, C. M. and Andrade-Montemayor, H. M. 2015. Nutritional composition, in vitro degradability and gas production of *Opuntia ficus indica* and four other wild cacti species. *Livestock Science* 12:42-54.
- Costa, C. T. F.; Ferreira, M. A.; Campos, J. M. S.; Guim, A.; Silva, J. L.; Siqueira, M. C. B.; Barros, L. J. A. and Siqueira, T. D. Q. 2016. Intake, total and partial digestibility of nutrients, and ruminal kinetics in crossbreed steers fed with multiple supplements containing spineless cactus enriched with urea. *Livestock Science* 188:55-60. <https://doi.org/10.1016/j.livsci.2016.04.008>
- Detmann, E. 2012. *Métodos para análise de alimentos*. Suprema, Visconde do Rio Branco.
- Dufreneix, F.; Faverdin, P. and Peyraud, J.-L. 2019. Influence of particle size and density on mean retention time in the rumen of dairy cows. *Journal of Dairy Science* 102:3010-3022. <https://doi.org/10.3168/jds.2018-15926>
- Lins, S. E. B.; Pessoa, R. A. S.; Ferreira, M. A.; Campos, J. M. S.; Silva, J. A. B. A.; Silva, J. L.; Santos, S. A. and Melo, T. T. B. 2016. Spineless cactus as a replacement for wheat bran in sugar cane-based diets for sheep: intake, digestibility, and ruminal parameters. *Revista Brasileira de Zootecnia* 45:26-31. <https://doi.org/10.1590/S1806-92902016000100004>
- Manella, M. Q.; Lourenço, A. J. and Leme, P. R. 2002. Recria de bovinos Nelore em pastos de *Brachiaria brizantha* com suplementação protéica ou com acesso a banco de proteína de *Leucaena leucocephala*: desempenho animal. *Revista Brasileira de Zootecnia* 31:2274-2282.
- NRC - National Research Council. 2007. *Nutrient requirement of small ruminants*. Washington, DC, USA.
- Nefzaoui, A. and Ben Salem, H. 2001. *Opuntia* spp.: a strategic fodder and efficient tool to combat desertification in the WANA region. p.73-90. In: Mondragón-Jacobo, C. and Pérez-González, S., eds. *Cactus (Opuntia spp.) as forage*. FAO Plant Production and Protection Paper 169. FAO, Rome.
- Oliveira, F. T.; Souto, J. S.; Silva, R. P.; Andrade Filho, F. C. and Pereira Júnior, E. B. 2010. Palma forrageira: Adaptação e importância para os ecossistemas áridos e semiáridos. *Revista Verde de Agroecologia e Desenvolvimento Sustentável* 5:27-38.
- Oliveira, V. S.; Santana Neto, J. and Valença, R. L. 2013. Características químicas e fisiológicas da fermentação ruminal de bovinos em pastejo–Revisão de literatura. *Revista Científica Eletrônica Medicina Veterinária* 20:1-21.
- Pinchak, W. E.; Min, B. R.; Malinowski, D. P.; Sij, J. W.; Gill, R. J.; Puchala, R. and Anderson, R. A. 2005. Re-evaluation of the frothy bloat complex in cattle grazing winter wheat in the Southern plains: evolution of a new integrated paradigm. *Proc. Gastrointestinal Function*. CGIF, Chicago, IL. 36p.
- Pinho, R. M. A.; Santos, E. M.; Oliveira, J. S.; Carvalho, G. G. P.; Silva, T. C.; Macêdo, J. F. S.; Corrêa, Y. R. and Zanine, A. M. 2018. Does the level of forage neutral detergent fiber affect the ruminal fermentation, digestibility and feeding behavior of goats fed cactus pear? *Animal Science Journal* 89:1424-1431. <https://doi.org/10.1111/asj.13043>
- Pressey, R.; Synhorst, S. H.; Bertram, J.; Allen, R. S. and Jacobson, N. L. 1963. Foaming properties of alfalfa and their relationship to bloat. *Journal of Animal Science* 22:970-978. <https://doi.org/10.2527/jas1963.224970x>
- Sáenz, C.; Sepúlveda, E. and Matsuhira, B. 2004. *Opuntia* spp. mucilage's: a functional component with industrial perspectives. *Journal Arid Environments* 57:275-290. [https://doi.org/10.1016/S0140-1963\(03\)00106-X](https://doi.org/10.1016/S0140-1963(03)00106-X)
- Satter, L. D. and Slyter, L. L. 1974. Effect of ammonia concentration on rumen microbial protein production *in vitro*. *British Journal Nutrition* 32:199-208. <https://doi.org/10.1079/BJN19740073>
- Silva, J. C.; Vêras, A. C.; Carvalho, F. R.; Ferreira, M. A.; Souza, E. O.; Barreto, L. G.; Lopes, L. A. and Silva, J. V. 2017. Nutritional value, performance, feeding behavior and serum biochemical profile of sheep fed with alfalfa hay replacing Bermuda grass (*Cynodon dactylon* (L.) Pers.) hay. *Chilean Journal Agricultural Research* 77:340-345. <https://doi.org/10.4067/S0718-58392017000400340>
- Silva, T. G. P.; Batista, A. M. V.; Guim, A.; Silva Júnior, V. A.; Carvalho, F. F. R.; Barros, M. E. G.; Sousa, D. R. and Silva, S. M. C. 2020. Histomorphometric changes of the fore-stomach of lambs fed with diets containing spineless cactus genotypes resistant to *Dactylopius* sp. *Tropical Animal Health and Production* 52:1299-1307. <https://doi.org/10.1007/s11250-019-02129-0>

Siqueira, M. C. B.; Ferreira, M. A.; Monnerat, J. P. I. S.; Silva, J. L.; Costa, C. T. F.; Conceição, M. G.; Andrade, R. P. X.; Barros, L. J. A. and Melo, T. T. B. 2017. Optimizing the use of spineless cactus in the diets of cattle: total and partial digestibility, fiber dynamics and ruminal parameters. *Animal Feed Science and Technology* 226:56-64. <https://doi.org/10.1016/j.anifeedsci.2016.12.006>

Sniffen, C. J.; O'Connor, J. D.; Van Soest, P. J.; Fox, D. G. and Russel, J. B. 1992. A net carbohydrate and protein system for evaluating cattle diets: II. Carbohydrate and protein availability. *Journal of Animal Science* 70:3562-3577.

Souza Filho, P. F.; Ribeiro, V. T.; Santos, E. S. and Macedo, G. R. 2016. Simultaneous saccharification and fermentation of cactus pearbiomass—evaluation of using different pretreatments. *Industrial Crops and Products* 89:425-433. <https://doi.org/10.1016/j.indcrop.2016.05.028>

Stintzing, F. C. and Carle, R. 2005. Cactus stems (*Opuntia spp.*): A review on their chemistry, technology, and uses. *Molecular Nutrition Food Research* 49:175-194. <https://doi.org/10.1002/mnfr.200400071>

Van Soest, P. J. 1994. *Nutritional ecology of the ruminant*. 2nd ed. Cornell University Press, Ithaca.

Wanapat, M. and Kang, S. 2013. Enriching the nutritive value of cassava as feed to increase ruminant productivity. *Journal of Nutritional Ecology Food Research* 1:262-269. <https://doi.org/10.1166/jnef.2013.1048>